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Immunologic and virologic findings in a bull chronically infected with noncytopathic bovine viral diarrhea virus

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Abstract
In February 1985, a 2-year-old bull with inappetence and weight loss of 1-week duration was examined by the field services staff of the Iowa State University College of Veterinary Medicine. The bull had a normal rectal temperature, decreased rumen activity, and loose feces and walked with a stiff gait. The hemogram was normal for hemoglobin concentration, PCV, and total plasma protein concentration, but the bull had a leukopenia which was primarily attributable to an absolute lymphopenia. Treatment consisted of antimicrobial drugs, an antidiarrheal medication, and a nonsteroidal anti-inflammatory and analgesic drug. After 3 weeks without improvement, the bull was admitted to the Iowa State University Large Animal Hospital.

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Comments
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Immunologic and virologic findings in a bull chronically infected with noncytopathic bovine viral diarrhea virus

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In February 1985, a 2-year-old bull with inappetence and weight loss of 1-week duration was examined by the field services staff of the Iowa State University College of Veterinary Medicine. The bull had a normal rectal temperature, decreased rumen activity, and loose feces and walked with a stiff gait. The hemogram was normal for hemoglobin concentration, PCV, and total plasma protein concentration, but the bull had a leukopenia which was primarily attributable to an absolute lymphopenia. Treatment consisted of antimicrobial drugs, an antidiarrheal medication, and a nonsteroidal anti-inflammatory and analgesic drug. After 3 weeks without improvement, the bull was admitted to the Iowa State University Large Animal Hospital.

On admission, the bull was depressed, thin, and reluctant to move. Rectal temperature was 39.7 C, the pulse rate was 84 beats/min, and the respiratory rate was 60 breaths/min. Although the rectum was empty, watery feces were passed during physical examination. The differential diagnoses included chronic bovine viral diarrhea (BVD), chronic laminitis, paratuberculosis, salmonellosis, and intestinal parasitism.

For 5 days, the bull was given oxytetracycline IV and phenylbutazone, propylene glycol, and probiotic paste orally. For the next 4 days, only oxytetracycline was given IM. The bull continued to have intermittent inappetance and watery diarrhea, and the bull's rectal temperature fluctuated from 39.2 C to 40.2 C. Hemograms indicated leukopenia with an absolute lymphopenia. Plasma protein concentration decreased from 7.0 g/dl at admission to the hospital to 5.7 g/dl 10 days later.

Examination of fecal specimens did not indicate intestinal parasite ova (flotation), *Salmonella* (bacteriologic culture), or BVD virus (virologic culture). The bull did not respond to an IV johnin test.[1] Neutralizing antibodies against BVD virus were not detected in serum samples (the cytopathic Singer isolate of BVD virus was used as the reference virus). A noncytopathic BVD virus was detected in serum samples, at a concentration of 10,000 cell culture infective units/ml. Noncytopathic BVD virus was isolated repeatedly from serum samples collected during a 1-month period, indicating persistent BVD virus infection. The clinical signs and isolation of BVD virus indicated that the bull had chronic BVD.

Because cattle with acute BVD develop depressed neutrophil function and bacteremia[2,3] and because we thought that the effects of chronic BVD on neutrophil function may be similar to that of acute BVD, the bull was given ascorbic acid and lithium carbonate for 10 days, beginning 1 week after antimicrobial treatment ended; lithium carbonate stimulates granulopoesis, and ascorbic acid enhances neutrophil function.[4,5] Ascorbic acid (40 mg/kg of body weight) was given SC once daily for 3 days and then once every other day; lithium carbonate (8.8 mg/kg) was given orally once daily.

Leukocyte response during the 10-day treatment was monitored by use of hemograms and by use of the following tests of cellular function: lymphocyte blastogenesis in response to mitogens (phytohemagglutinin [PHA], concanavalin A, and pokeweed mitogen), random migration of polymorphonuclear leukocytes (PMN) under agarose, ingestion of ^{125}I-labeled *Staphylococcus aureus* by PMN, cytochrome C reduction by PMN, iodination by PMN, and PMN antibody-dependent cell-mediated cytotoxicity.[6,7] The cellular function assays were performed on the day before treatment began, on the day that treatment began, and on treatment days 4 and 7. For each assay, leukocytes from 3 healthy, nontreated, adult steers were similarly assayed. Hemograms were performed on the day before treatment began, on the day treatment began, and on treatment days 4, 7, and 10.

Treatment with ascorbic acid and lithium carbonate did not statistically change the numbers or function of neutrophils or lymphocytes. However, in some function assays, mean responses of leukocytes from the bull ($n = 4$) were significantly different from those of leukocytes from healthy steers (controls, $n = 12$). The mean ($\pm$ SEM) lymphocyte blastogenesis in response to PHA was significantly

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($P<0.05$) lower for the bull (8,200 ± 3,600 counts/min) than for the control steers (24,500 ± 4,600 counts/min). Compared with neutrophils from the steers, neutrophils from the bull had a significantly ($P<0.01$) greater ability to ingest *S aureus* (41 ± 4.7% ingestion vs 56.9 ± 10.9% ingestion) and were significantly less able to iodinate protein (31.1 ± 3.2 nmol of NaI/10^7 PMN/hr vs 11 ± 1% nmol of NaI/10^7 PMN/hr). Other evaluated determinants of lymphocyte and neutrophil function were comparable between the bull and control steers.

The bull's condition deteriorated during treatment with ascorbic acid and lithium carbonate. The day after this treatment regimen ended, the bull was euthanatized. At necropsy, necrotic areas were seen at the tips of papillae of the buccal mucosa. Epithelial indentations (indicating healed erosions) were seen on the tongue and on the ruminal pillar mucosa. Macroscopic mucosal lesions were not seen in the intestines, but fibrinous casts were found in the jejunum and colon. Retropharyngeal lymph nodes and all mesenteric lymph nodes were edematous.

Microscopically, mild to moderate villous atrophy was seen in the jejunum and ileum, with edema of the lamina propria and an absence of Peyer's patches. Glands of the ileocecal junction and proximal portion of the colon were dilated and had little to no periglandular lymphoid tissue. The colonic mucosa appeared to be atrophied. Secondary lymphoid follicles were not found in retropharyngeal or mesenteric lymph nodes or in the spleen, and a distinction was not found between the cortex and medulla of the thymus.

Virus isolation was attempted from spleen specimens. Virus neutralization was done, using the immunoglobin (Ig)G fraction of serum collected immediately before euthanasia.[8,9] A noncytopathic BVD virus was isolated from the spleen. Neutralizing activity against 2 cytopathic BVD viruses was detected in the 1:64 and 1:4 dilutions of the IgG fraction of the serum sample. Neutralizing activity against the noncytopathic BVD virus that persistently infected the bull and against another noncytopathic BVD virus was detected at the 1:4 dilution of the IgG fraction. Neutralizing activity was not detected against 1 other noncytopathic or 3 other cytopathic BVD viruses.

Mucosal disease and chronic BVD are thought to develop only in cattle with a congenital, persistent infection with noncytopathic BVD virus.[10–12] Cattle persistently infected with certain noncytopathic BVD viruses develop mucosal disease after becoming superinfected under experimental conditions with certain cytopathic BVD viruses.[11,12] In mucosal disease, the disease is severe, death occurs within a few days, and noncytopathic and cytopathic BVD viruses can be isolated from the spleen.[8,12] Thus, the pathogenetic mechanism for mucosal disease involves simultaneous infection by 2 types of BVD virus. However, all combinations of noncytopathic and cytopathic BVD viruses do not induce mucosal disease. Under experimental conditions, cattle persistently infected with certain noncytopathic BVD viruses do not develop overt disease when they are inoculated with certain cytopathic BVD viruses, and virus-neutralizing antibodies specific for the cytopathic BVD virus are produced.[9,13]

Chronic BVD differs from mucosal disease in that the disease is less severe and longer in duration,[14] but is simlar to mucosal disease in that affected cattle have a congenital, persistent noncytopathic BVD virus infection.[10–12] In cattle that die from chronic BVD, noncytopathic BVD virus is isolated from the spleen, but cytopathic BVD virus frequently is not isolated.[8] Mucosal disease has been induced experimentally.[11,12] The pathogenetic mechanism of chronic BVD may be similar to that of mucosal disease in that simultaneous infection with certain noncytopathic and cytopathic BVD viruses is required to induce chronic BVD. The severity of disease (mucosal disease, chronic BVD, or no overt disease) might depend on antigenic or other relationships between noncytopathic and cytopathic BVD viruses.[9]

The bull of the present report was from a herd that did not have a history of clinical BVD and in which modified-live cytopathic BVD virus vaccines had not been used. The bull had been vaccinated with killed cytopathic BVD virus (BVD-Singer) in the spring of 1984 and had been revaccinated with the same vaccine after onset of clinical disease in February 1985. Thus, the only known exposure of the bull to a BVD virus other than to the noncytopathic BVD virus that induced the persistent infection was to the killed cytopathic BVD vaccine virus.

The killed cytopathic vaccine virus may have been the only heterologous BVD virus to which the bull was exposed, because highly specific neutralizing antibodies (1:64 titer) against the vaccine virus were detected, but little or no neutralizing activity was detected against several other BVD virus isolates. The insidious onset of disease that may have been induced by vaccination with the killed virus might indicate that an immunologically mediated reaction is an important component of the pathogenetic mechanism for chronic BVD. If the killed vaccine virus induced chronic BVD, cytopathic BVD virus probably would not have been isolated from the spleen.

The bull may have been naturally exposed to a field strain of cytopathic BVD virus that was antigenically distinct from the BVD viruses used in the neutralization tests. If so, neutralizing antibodies specific for the field strain of cytopathic BVD virus would have been produced. Antibody clearance of the field strain of virus from the bull may explain our failure to isolate cytopathic BVD virus from the bull's spleen. A high titer of BVD virus-neutralizing antibodies probably would not have been detected because of antigenic differences between the field virus and the viruses used in the neutralization tests.

Other possible causes of chronic BVD in the bull include an idiopathic sequel of persistent infection

with noncytopathic BVD virus[10] or superinfection with a heterologous noncytopathic BVD virus. In either case, cytopathic BVD virus would not be isolated from the spleen, and neutralizing antibodies specific for the vaccine virus probably would not be involved in the disease process.

Although we did not determine what induced the clinical disease in the bull of the present report, the bull did produce neutralizing antibodies highly specific for a single BVD virus (BVD-Singer). Experimentally, neutralizing antibodies specific for certain BVD viruses have been produced in persistently infected cattle after exposure to cytopathic BVD virus.[9,13] Neutralizing antibodies specific for cytopathic BVD virus have been reported in cattle with chronic BVD or postvaccinal mucosal disease.[15] The bull of the present report repeatedly had a depressed lymphocyte blastogenic response to PHA stimulation. Depressed lymphocyte response to PHA stimulation has been detected in clinically healthy cattle persistently infected with noncytopathic BVD virus and in some cattle with chronic BVD.[15–17] Depressed iodination and enhanced bacterial ingestion by PMN was repeatedly detected in the bull of the present report.

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